AD-A073 065 MASSACHUSETTS UNIV AMHERST DEPT OF PLANT PATHOLOGY F/G 6/1
CYCLIC NUCLEOTIDES AND PECTIC ENZYME SYNTHESIS.(U)
AUG 79 M S MOUNT DAAG29-76-G-0273

UNCLASSIFIED ARO-13450.4-L NL

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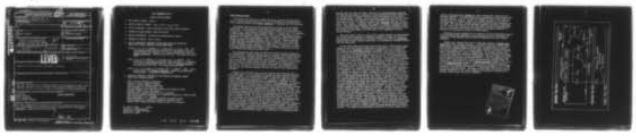

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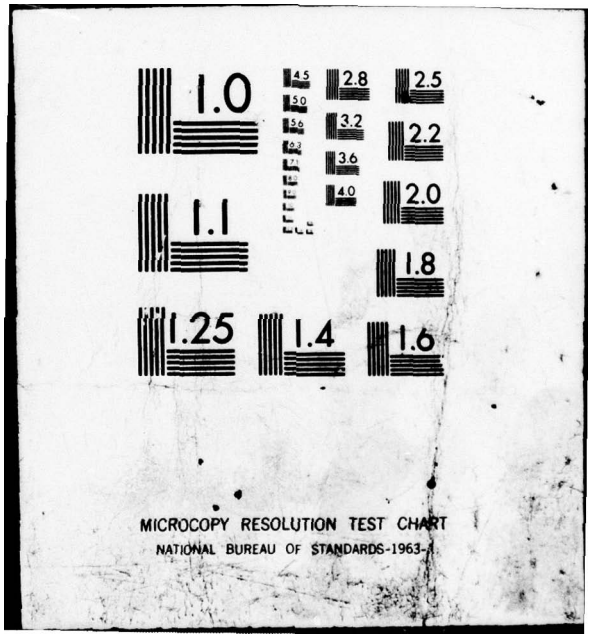
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MICROCOPY RESOLUTION TEST CHART
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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: (19) 13450.4-L (18) ARO
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): (6) Cyclic Nucleotides and Pectic Enzyme Synthesis
5. TYPE OF REPORT & PERIOD COVERED: (9) Final Report, 1 Jun 76 - 31 May 79
6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s): (10) Mark S. Mount
8. CONTRACT OR GRANT NUMBER(s): (15) DAAG29-76-G-0273

9. PERFORMING ORGANIZATION NAME AND ADDRESS
University of Massachusetts
Amherst, Massachusetts 01003
Dept. of Plant Pathology, Nav

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

11. CONTROLLING OFFICE NAME AND ADDRESS
U. S. Army Research Office
P. O. Box 12211
Research Triangle Park, NC 27709
(12) 6 pp (11)

12. REPORT DATE: Aug 79
13. NUMBER OF PAGES: 5

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report): Unclassified
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

LEVEL II

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

DDC RECEIVED AUG 24 1979

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

nucleotides
enzyme synthesis
cyclic nucleotides
pectic enzyme synthesis
Erwinia carotovora

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report summarizes the results of a study to elucidate the possible role of cyclic nucleotides in the regulation of pectic enzyme synthesis from Erwinia carotovora, a vegetable soft rot bacterium.

411349

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

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AD A073065

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FINAL ~~PROGRESS~~ REPORT

(FORTY COPIES REQUIRED)

1. ARO PROPOSAL NUMBER: 13450 L

2. PERIOD COVERED BY REPORT: June 1, 1976 - May 31, 1979

3. TITLE OF PROPOSAL: Cyclic Nucleotides and Pectic Enzyme Synthesis

4. CONTRACT OR GRANT NUMBER: DAAG-29-76-G-0273

5. NAME OF INSTITUTION: University of Massachusetts, Amherst

6. AUTHOR(S) OF REPORT: Mark S. Mount

7. LIST OF MANUSCRIPTS SUBMITTED OR PUBLISHED UNDER ARO SPONSORSHIP DURING THIS PERIOD, INCLUDING JOURNAL REFERENCES:

Hubbard, J. P., J. D. Williams, R. M. Niles and M. S. Mount. 1978. The relationship between glucose repression of endo-polygalacturonate trans-eliminase and adenosine 3',5'-cyclic monophosphate levels in Erwinia carotovora. Phytopathology 68:95-99. (25 copies previously sent)

Mount, M. S., P. M. Berman, R. P. Mortlock, and J. P. Hubbard. 1979. Regulation of endo-polygalacturonate trans-eliminase in an adenosine 3',5'-cyclic monophosphate-deficient mutant of Erwinia carotovora. Phytopathology 69:117-120. (25 copies previously sent)

Stack, J. P., M. S. Mount, P. M. Berman, and J. P. Hubbard. 1980. Pectic enzyme complex from Erwinia carotovora: A model for pathogenesis. Phytopathology. (Accepted for publication)

8. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED DURING THIS REPORTING PERIOD:

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Final Progress Report

The overall objective of my research during the previous granting period was to elucidate the possible role of cyclic nucleotides in the regulation of pectic enzyme synthesis from Erwinia carotovora, a vegetable soft rot bacterium.

We observed that both the rate of growth and the rate of pectic enzyme accumulation in this bacterium on minimal salts was affected by the carbon source used. Cells growing on sodium polypectate (NaPP) or on glucose grew at a similar rate. Cells growing on glycerol grew at a reduced rate. The synthesis of endo-polygalacturonate trans-eliminase (PGTE), in induced culture, is repressed in the presence of glucose, but not by glycerol. Exogenously supplied adenosine 3',5'-cyclic monophosphate (cAMP) reverses repression of PGTE synthesis, provided the enzyme substrate, NaPP, is present. Various analogues of cAMP and other nucleotide derivatives such as dibutyrl 3',5'-cyclic AMP, 5'-AMP, adenosine, 5'-ATP, 8-bromo 3',5'-cAMP, and 8' methylthio 3',5'-cAMP failed to reverse glucose repression of PGTE synthesis. When cGMP was added to induced cultures along with glucose, levels of PGTE activities were below those observed when glucose was added alone.

Also measured were the levels of cAMP under various cultural conditions. The authenticity of cAMP was demonstrated by a specific cAMP phosphodiesterase. It was found that a decrease in cAMP could be correlated with glucose repression and a decrease in PGTE synthesis. As PGTE accumulates in induced cultures, the specific concentration of cAMP increases. Although it was observed that the levels of cAMP in glycerol grown cultures were relatively high, the specific activity of PGTE did not increase until NaPP was supplemented to the culture. If glucose was added along with NaPP to such a glycerol grown culture, the PGTE activity remained low and the relative cAMP concentration decreased.

Although the biochemical data strongly suggested that PGTE synthesis is under cAMP control, it was necessary to confirm the PGTE regulatory mechanism by genetic means. The conventional methods for obtaining adenylate cyclase mutants (cya) (lacks the conversion of ATP to cAMP) in E. coli were unsuccessful in manufacturing cya mutants in E. carotovora. Therefore we had to devise a new method for obtaining these mutants in E. carotovora. We selected a β-galactosidase constitutive mutant (β-gal^c) by growing nitrosoguanidine (NG) mutated cells on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). When X-gal is hydrolyzed by β-galactosidase, a deep blue dye is released. In the absence of the inducer, isopropyl-β-D-thiogalactoside (IPTG), a dark blue colony would indicate constitutive levels of enzyme. One such deep blue colony was cloned and exhibited high levels of β-galactosidase activity in the presence or absence of IPTG. To obtain cAMP-deficient mutants, this β-gal^c mutant was treated with NG and colonies selected which lost the ability to produce a blue color on X-gal medium in the absence of IPTG. These mutants were then tested for their ability to grow on various carbohydrates such as α-lactose, L-arabinose, D-galactose, D-mannitol, L-rhamnose, D-xylose, raffinose, D-cellobiose, glycerol, D-ribose and sodium polypectate (NaPP). The mutant EC1491 demonstrated a positive growth response to the addition of cAMP to the growth media. The catabolism of D-ribose, D-mannitol, and D-glucose was not affected in the cAMP-deficient mutant. The synthesis of the inducible pectic

enzyme, PGTE, was under the direct control of the cAMP regulatory mechanism. The cAMP-deficient mutant, growing on a casein hydrolyzate-minimal salts medium, produced only low levels of PGTE, even in the presence of the inducer, sodium polypectate. However, when both sodium polypectate and cAMP were added, the levels of PGTE were elevated to levels comparable with those of the induced parent strain. In addition mutants of E. carotovora have been isolated which do not respond to exogenous cAMP supplied to various carbohydrate growth media. These have been designated, therefore, as potential catabolite repressor protein mutants (crp). Experiments are currently being performed to prove the validity of these mutants as they relate to the regulation of pectic enzyme synthesis.

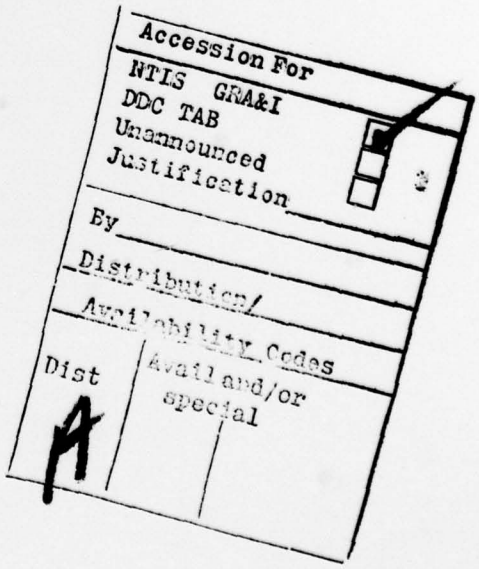
Since the current concept is that pectic enzymes released by phytopathogens are the key factors involved in enzymatic hydrolysis of plant cell walls, it was necessary to re-evaluate all the pectic enzyme types which E. carotovora produces and how these enzymes interrelate to the pathogenic processes of plant cell wall hydrolysis.

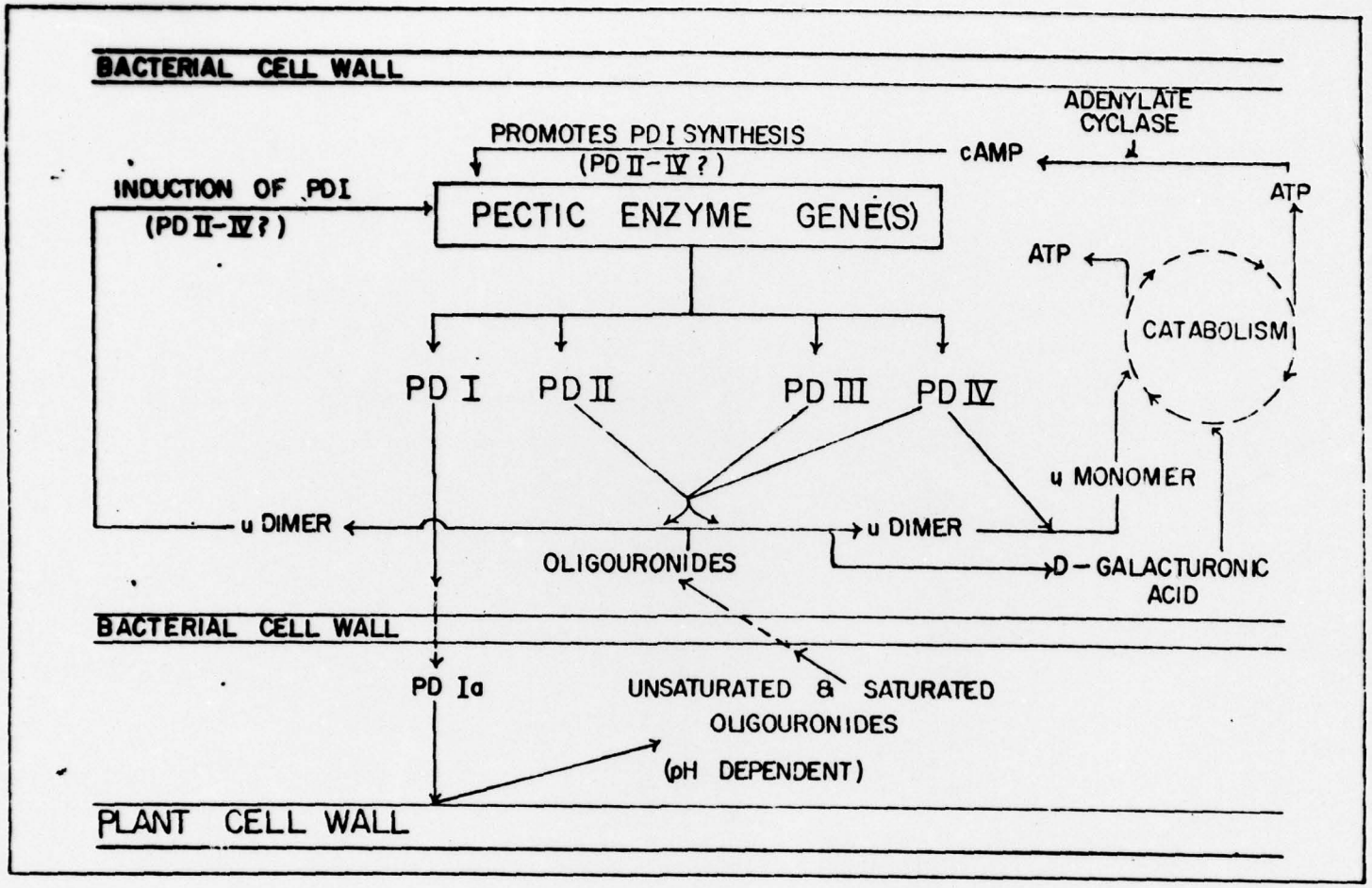
We have fully characterized the intracellular pectic enzymes from E. carotovora and have made several significant new discoveries. We observed that our isolate of E. carotovora synthesizes a minimum of 4 pectic enzymes; an endo-polygalacturonate trans-eliminase (endo-PGTE = PDI) with an isoelectric point of 9.4, an exo-PGTE (PDII) with a pI of 8.0 which possesses some endo-like properties, an exo-PGTE (PDIII) with a pI of 6.3, and an oligogalacturonate trans-eliminase (OGTE - PDIV). Only PDI (pI 9.4) is capable of becoming extracellular and macerating the plant cell walls. The other pectin depolymerases (PDII-IV) remain within the bacterial cells and further hydrolyze the breakdown products of the PDI reacted polysaccharides. The reaction products, as determined by paper chromatography, all differ according to the enzyme used and it appears that the ultimate product from the action of these 4 enzymes together working in conjunction with each other is an unsaturated galacturonic acid (monomer). PDI, which macerates cell walls, is incapable of producing the unsaturated monomer by itself. Preliminary data indicate that the unsaturated monomer is capable of induction of PDI but is not as effective an inducer as unsaturated digalacturonic acid (dimer) (a reaction product of PDIII). Also of great importance is the fact that we have found PDI in a reaction mixture under different ion conditions and pH behaves as a hydrolase rather than a trans-eliminase enzyme making only saturated breakdown products. We have not proven definitively whether PDI is a single enzyme that possesses dual enzymatic properties (hydrolase and trans-eliminase) or whether it is two separate enzymes with similar physical properties. Several lines of evidence we have obtained indicate the latter may be true. (1) After isoelectric focusing, if the PDI complex is subjected to Sephracryl S-200 gel filtration, many times the hydrolase activity is irreversibly lost, while the trans-eliminase activity is always recoverable. (2) Upon purification of PDI the specific activities of each enzyme activity vary (trans-eliminase is always produced in higher amounts). (3) Saturated galacturonic acid does not induce the synthesis of the trans-eliminase enzyme, however the saturated monomer is an effective inducer of hydrolase activity. The above observations are circumstantial, but do, however, suggest two enzymes. We are currently attempting to demonstrate visually two protein bands on polyacrylamide disc gel electrophoresis. The hydrolytic-transeliminase properties of the PDI complex may be

a key to understanding why Erwinia has such a diverse host range and is an effective pathogen. This dual enzyme activity phenomenon is also evident with the PDIV complex. As of yet we have not attempted to fully characterize (or separate) the PDIV enzymes. Figure 1 is a model proposed by us to outline the significance of these findings upon which further work and evaluation can be based. This bacterium has the necessary enzymatic machinery to depolymerize the pectic acid fraction of plant cell walls, utilize the breakdown products as a food source for energy, and generate the necessary molecules to maintain the synthesis and activity of these enzymes.

Additional studies initiated in our laboratory involved determining whether extra-chromosomal DNA (plasmids) was the location for any of the genetic determinants of pathogenesis and/or regulatory mechanisms for pectic enzyme synthesis.

Plasmids were isolated from five of twenty-five strains of Erwinia carotovora which were collected worldwide. An isolate E-131 from tobacco hollow stalk showed a large plasmid of approximately 55 megadaltons by co-electrophoresis with standard plasmids of known size, and strain E-130 contained an even larger plasmid species. An onion isolate, EC-527, contained two plasmid bands of approximately 8.4 and 3.9 megadaltons. Two Florida isolates from lettuce and tomato each possessed multiple small bands of plasmid DNA ranging in molecular weights of 3.4 to 10.2 megadaltons. Two other strains from South America showed an unusual "constricted plasmid" in the 3-4 megadalton range. One of these strains produced a potent bacteriocin-like substance which was inhibitory to the growth of all other Erwinia carotovora strains as well as to several Escherischia coli strains. As all strains were strongly pectolytic but of variable plasmid content, no significance of plasmids to pectic enzyme synthesis of the pathogen was observed. Of particular interest to us is the bacteriocin producing strain. We intend to fully define the genetic and biochemical parameters of this lethal compound and evaluate its potential as a possible control agent for soft rot diseases.




BACTERIAL CELL WALL
ADENYLATE CYCLASE
PROMOTES PDI SYNTHESIS (PD II-IV?)
cAMP
ATP
INDUCTION OF PDI (PD II-IV?)
PECTIC ENZYME GENE(S)
ATP
CATABOLISM
PD I
PD II
PD III
PD IV
u MONOMER
u DIMER
u DIMER
OLIGOURONIDES
D-GALACTURONIC ACID
BACTERIAL CELL WALL
PD Ia
UNSATURATED & SATURATED OLIGOURONIDES
(pH DEPENDENT)
PLANT CELL WALL